

MICROBIAL TRANSFORMATIONS OF TRU AND MIXED WASTES: ACTINIDE SPECIATION AND WASTE VOLUME REDUCTION.

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RESEARCH OBJECTIVES

The overall goals of this research project are to determine the mechanism of microbial dissolution and stabilization of actinides in Department of Energy's (DOE) TRU wastes, contaminated sludges, soils, and sediments. This includes (i) investigations on the fundamental aspects of microbially catalyzed radionuclide and metal transformations (oxidation/reduction reactions, dissolution, precipitation, chelation); (ii) understanding of the microbiological processes that control speciation and alter the chemical forms of complex inorganic/organic contaminant mixtures; and (iii) development of new and improved microbially catalyzed processes resulting in immobilization of metals and radionuclides in the waste with concomitant waste volume reduction.

RESEARCH PROGRESS AND IMPLICATIONS

This is a final report which summarizes work after 3 years of a 3 year project.

I. REDUCTIVE DISSOLUTION OF PLUTONIUM

Anaerobic microbial dissolution of Pu. We investigated the biotransformation of ^{242}Pu -nitrate by the anaerobic bacterium *Clostridium* sp. Adding

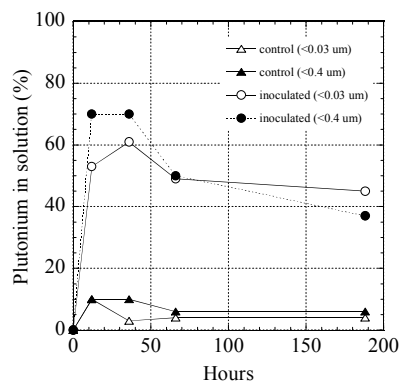


Fig. 1. Dissolution of polymeric Pu species by the activity of *Clostridium* sp.

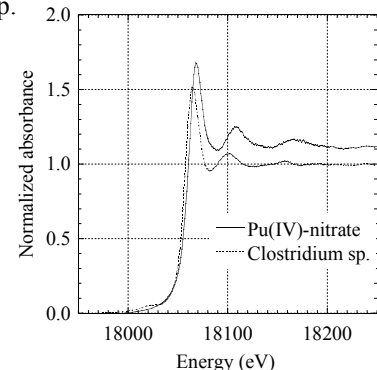


Fig 2. XANES analysis of Pu before (i-) and following (---) anaerobic microbial activity.

nitrate by the anaerobic bacterium *Clostridium* sp. Adding 1×10^{-7} M ^{242}Pu (IV)-nitrate had no effect upon its growth and metabolism of glucose. Ninety percent of the added Pu in uninoculated growth medium (control) was removed by $0.4 \mu\text{m}$ filtration, and most likely existed as $\text{Pu}(\text{OH})_4$ at pH 6.2 due to hydrolysis and polymerization reactions. The growth of *Clostridium* sp. lowered the Eh to -180 mV and the pH from 6.2 to 2.8, concomitant with the production of the organic acids, acetic and butyric (12 and 17 mM, respectively), and carbon dioxide (225 μmol). After 14h of growth, 70% of the Pu passed through a $0.4 \mu\text{m}$ filter and 55% through a $0.03 \mu\text{m}$ filter. This suggests that a soluble form of Pu was present and not a polymeric form.

Pu Speciation. Reduction of Pu(IV) to Pu(III). Solvent extraction of the growth medium using thenoyltrifluoroacetone (TTA) at pH 0 and pH 4 confirmed a decrease in polymeric form of Pu and an increase in the soluble fraction suggesting the presence of Pu^{3+} (data not shown). The Eh of the medium was highly reducing (-180 eV). X-ray absorption near edge spectroscopy (XANES) analysis of the culture at the Pu L_{III} edge (18.057 keV) confirmed the oxidation state as Pu^{3+} (Figure 2). Solvent extraction of each treatment showed a

dramatic increase in the amount of Pu found in the fraction indicative of the soluble Pu species. The organic fraction at pH 4, indicative of +3, +4, and +6 Pu oxidation states, increased from 74 dpm/ml in the uninoculated growth medium to 575 dpm/ml in the presence of unfiltered spent medium. The organic fraction at pH 0, indicative of Pu⁴⁺ only, increased less dramatically from 46 dpm/ml in the uninoculated growth medium to 224 dpm/ml in the unfiltered spent medium. These results confirm that Pu is solubilized by *Clostridium* sp. following its reduction to Pu³⁺.

Mechanisms of Pu Dissolution. Pu biotransformation by direct or indirect action of *Clostridium* sp. was determined. Indirect mechanisms due to metabolite production, as well as, Eh and pH of the medium was determined in the absence of bacterial cells using (i) uninoculated pre-reduced, autoclaved growth medium, (ii) pre-reduced synthetic spent medium containing

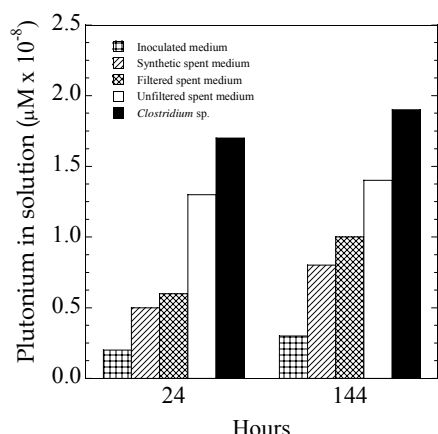


Figure 3. Effect of microbial extracellular metabolic products on solubilization of Pu(IV).

organic acids in the proportions found at logarithmic growth phase, (iii) unfiltered cell-free spent medium obtained from a 24-h old culture, and (iv) filtered cell-free spent medium (0.45 μm). Direct action was determined in the presence of growing bacterial cells. The extent of Pu dissolution by various treatments is shown in Figure 3. At 144-h, the uninoculated growth medium showed 15% of the Pu remained in solution following filtration through a 0.45 μm filter. In the synthetic spent medium 41% of the Pu was solubilized, in the filtered spent medium 33% of the Pu was in soluble form, and the unfiltered spent medium had 54% of the Pu in soluble form. This compares with 74% of the Pu passing through the filter in the presence of bacteria

II. BIODEGRADATION OF ORGANIC LIGANDS IN TRU WASTES

Isosaccharinic acid. The presence of organic ligands in radioactive wastes is a major concern because of their potential for increasing the transport of radionuclides from disposal

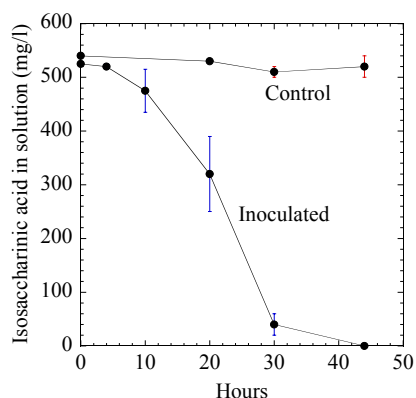


Fig 4. Biodegradation of ISA by an aerobic soil bacterial isolate.

sites. Biotransformation of radionuclides complexed with organic ligands should precipitate the radionuclides and retard their migration. Isosaccharinic acid (ISA) is a degradation product of cellulose when exposed to radiation at high pH. ISA is also known to form strong complexes with actinides (An) and may enhance their mobility from waste sites. Biodegradation of An-ISA complexes should retard the mobility of actinides. We investigated the biodegradation of ISA by an aerobic bacterium (gram-positive rod) isolated by enrichment culture technique. Degradation of ISA by the bacterial isolate is shown in Figure 4. Currently, we are investigating the biotransformation of the metal- and radionuclide-ISA complexes under both aerobic and anaerobic conditions.

Pu-citrate. *Structural determination of Pu: citric acid complex.* The molecular association of Pu(IV) with citric acid was determined so that a structure-function relationship

could be obtained. The XANES spectra for Pu(IV)-nitrate and Pu-citrate complexes are presented in Figure 5A. The first derivative of the absorption-edge energy for the Pu-citrate complex is at 18060 eV. This is identical to that for the Pu(IV)-nitrate standard, thereby confirming the tetravalent oxidation state of the Pu-citrate complex. Figure 5B and 5C shows the k^3 -weighted ($2.5\text{--}12.5 \text{ \AA}^{-1}$) raw EXAFS spectrum and the Fourier transform for the Pu-citrate complex. The inner-sphere coordination number for Pu is 10.0 and includes three distinct O shells. There are 5.0 ± 1.2 O's at $2.26 \pm 0.02 \text{ \AA}$, 3.5 O's at $2.41 \pm 0.02 \text{ \AA}$, and 1.5 ± 0.8 O atoms at $2.69 \pm 0.02 \text{ \AA}$. The atoms closest to the Pu at 2.26 \AA are due to the presence of water and other uncoordinated oxygens. The 3.5 O atoms at 2.41 \AA are coordinated to the Pu through the carboxylate oxygens of two citric acid molecules. The O atoms at 2.69 \AA are most probably due to coordination with the α -hydroxyl oxygens of citric acid. No Pu-Pu interaction typical for Pu(IV)-hydroxo complexes is observed. Based upon this analysis the best configuration for the molecule consists of a mononuclear biligand complex.

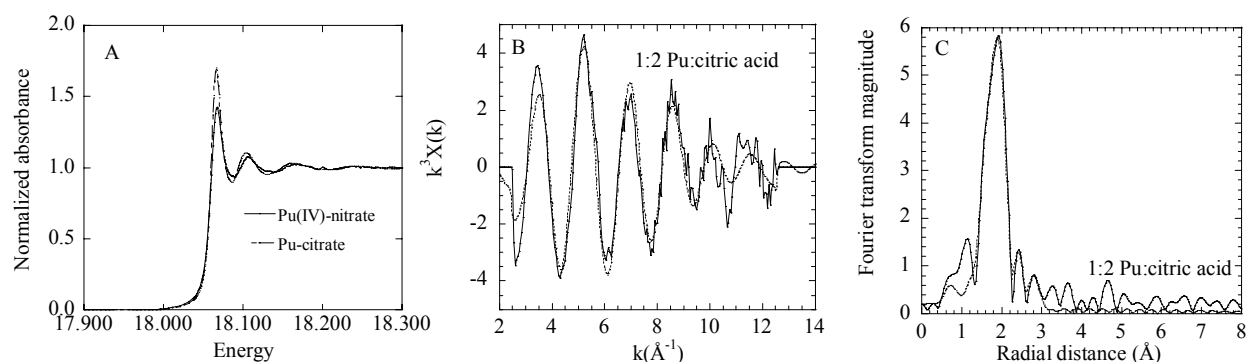


Figure 5. XANES spectrum for Pu(IV)-nitrate and Pu-citrate complex (A); and k^3 -weighted data (B) and Fourier-transformed EXAFS spectrum (C) for Pu: citric acid complex. Experimental data (-); fitted data (---).

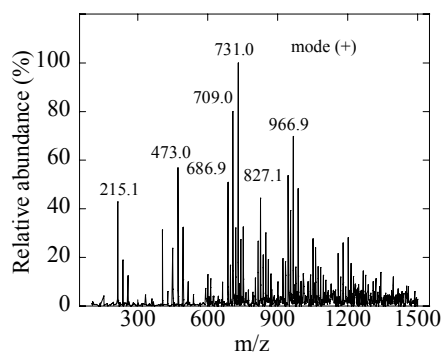


Figure 6. Electrospray ionization (ESI-MS) spectra produced by Pu-citrate complex.

Figure 6 shows the ESI-MS spectra for singly charged positive ions peaks of $1.6 \times 10^{-4} \text{ M}$ Pu-citrate complex. The dominant peaks are due to the formation of a monomeric Pu-citrate complex at m/z 473.0 $[\text{Pucit}(\text{H}_2\text{O})\text{Na}]^+$ (Figure 7A) and biligand complex formation is indicated by the presence of peaks at m/z 686.9 $[\text{Pu}(\text{H}_2\text{cit})_2\text{NO}_3]^+$, m/z 709.0 $[\text{Pu}(\text{Hcit})(\text{H}_2\text{cit})\text{NaNO}_3]^+$, and m/z 731.0 $[\text{Pu}(\text{Hcit})_2\text{Na}_2\text{NO}_3]^+$ (Figure 3B). The presence of a dimeric Pu complex is noted at m/z 966.9 $[\text{Pu}_2(\text{Hcit})(\text{cit})(\text{NO}_3)_2]^+$ (Figure 7C). The m/z values are considered accurate to within 1 or 2 amu's. Based upon the

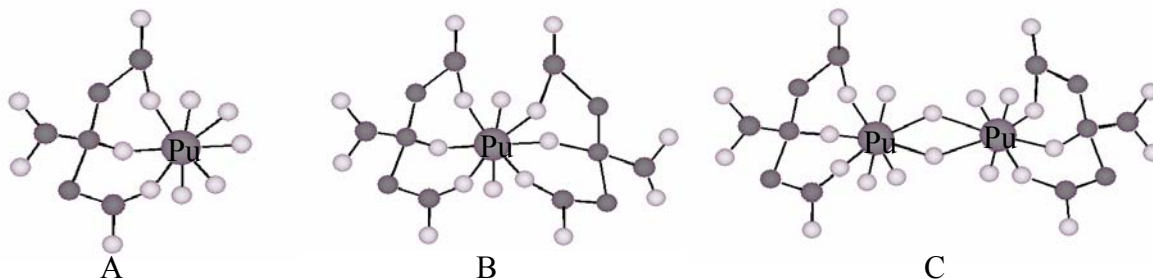


Figure 7. Proposed structures at pH 6 for the monoligand 1:1 Pu: citric acid complex (A); biligand 1:2 Pu: citric-acid complex $[\text{PuO}(\text{cit})_2]^4$ (B); and dimeric 2:2 Pu: citric acid complex (C). The open circles represent oxygen and the filled circles represent carbon atoms.

speciation calculations, X-ray absorption spectra, and LC-MS data, we propose a biligand $[\text{Pu}(\text{cit}_2)]$ complex structure, similar to that suggested by Metivier and Guillaumont (Figure 7B).

Biodegradation of citric acid and Pu-citrate complexes. Figure 8A depicts the rate and extent of citrate degradation in samples containing 10^{-6} M and 10^{-8} M Pu present as Pu-citrate complexes at an ionic strength of 0.18 M. Citric acid (10^{-4} M) in the absence of Pu was metabolized completely at a rate of $4.9 \mu\text{M/h}$. With 10^{-6} and 10^{-8} M Pu present as the Pu-citrate

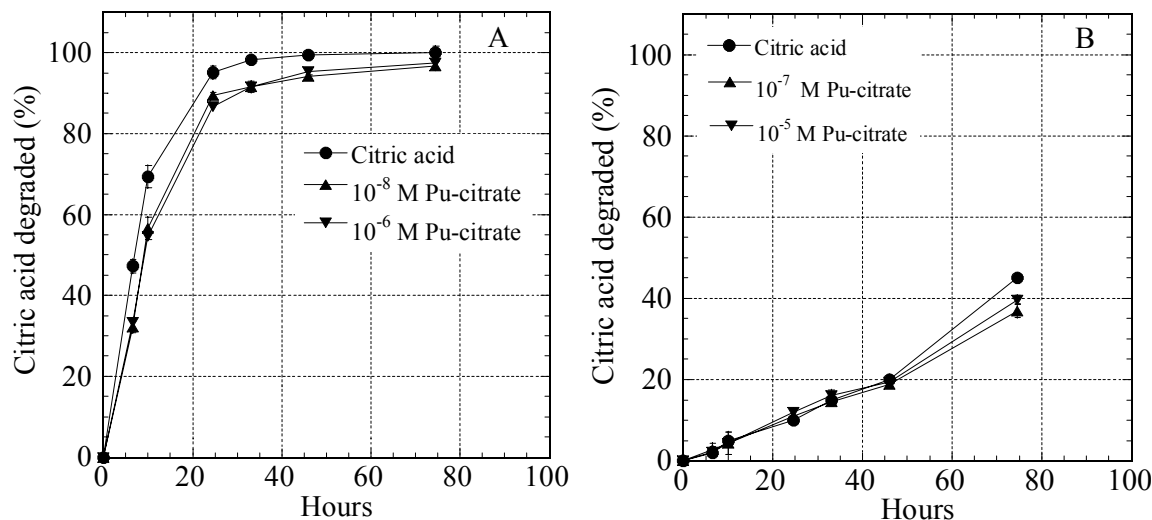


Figure 8. Effect of adding ^{242}Pu on citrate metabolism at an ionic strength of 0.18 M (A) and ionic strength 0.9 M (B).

complex we observed a slight decline on the rate and extent of citrate degradation in comparison to the sample lacking Pu. In both samples, citrate was degraded $>96\%$, and at the rate of $4.0 \mu\text{M/h}$ and $3.8 \mu\text{M/h}$, respectively. Increasing the ionic strength of the medium affected citrate metabolism. In high ionic-strength medium (0.9 M) containing 10^{-7} and 10^{-5} M Pu present as Pu-citrate complex, the rate and extent of citrate degradation was much lower at $0.8 \mu\text{M/h}$ in the 10^{-7} and 10^{-5} M Pu treatments with an overall degradation of 37% and 35%, respectively (Figure 8B). Without Pu, there was a slight increase in degradation to 45% of the total citric acid concentration.

Table 1. Speciation of Pu following 70-h incubation with *P. fluorescens*.

Pu			
Analysis	Oxidation state	Total activity (dpm)	Total extracted (%)
<u>10⁻⁶ M Pu</u>			
TTA _{org} (pH 4)	+3,+4,+6	64.5±3.5	30±5
TTA _{aq} (pH 4)	+5, polymer	154±17	70±11
TTA _{org} (pH 0)	+4	66±12	24±5
TTA _{aq} (pH 0)	+3,+5,+6, polymer	206±10	76±4
<u>10⁻⁵ M Pu</u>			
TTA _{org} (pH 4)	+3,+4,+6	501±44	19±9
TTA _{aq} (pH 4)	+5, polymer	2110±220	81±10
TTA _{org} (pH 0)	+4	443±18	20±4
TTA _{aq} (pH 0)	+3,+5,+6, polymer	1740±180	80±10

Speciation of plutonium before and after aerobic bacterial activity.

Thenoyltrifluoroacetone (TTA) was used to determine the oxidation state of Pu in the unfiltered samples following 70 hours of bacterial activity. Extraction of the low ionic strength sample containing 10^{-6} M Pu at pH 4. showed 70% of the Pu was present

as polymeric form with the remainder most probably as Pu^{4+} (Table 1). Although the TTA extraction also suggests the presence of PuO_2^+ , it is more likely that the tetravalent form is present as indicated by the XANES data. Extraction of the medium at pH 0 reveals that the predominant form of Pu (76%) is the polymer species.

Extracting the high ionic strength 10^{-5} M Pu into the aqueous phase at pH 4 indicated that Pu was predominantly polymeric, similar to the 10^{-6} M Pu sample. The speciation at pH 0 is similar to the results obtained at pH 4, with the polymeric species comprising 80% of the total Pu in the sample. The predominance of polymeric form of Pu in both treatments is most probably due to the biodegradation of citric acid which was almost completely metabolized at 10^{-6} M Pu and 40% biodegraded in the 10^{-5} M Pu treatments. In addition, the higher concentration of Pu in the 10^{-5} M sample may enhance polymer formation..

Tetravalent plutonium forms a 1:1 Pu: citric acid ($K=10^{19}$) complex and a 1:2 Pu: citric acid complex ($K=10^{34}$). In the presence of excess citric acid, their solubility and Pu(IV) oxidation state are stable. At pH 6.5, we identified a monomeric [Pucit] species, two forms of biligand [Pucit₂] species, and a dimeric [Pu₂cit₂] species of tetravalent plutonium with citric acid. Speciation calculations and ESI-MS show that the biligand complex is the predominant form. In addition, analysis of the complex over 100-h demonstrated that citric acid inhibits polymer formation. Although the Pu(IV)-citrate complex is stable in the absence of microbial activity, adding bacteria to the 1:100 and 1:10000 Pu(IV)-citrate complexes resulted in the retention of Pu species at 20-43% by the 0.4 μm (biomass associated)-filter, and 27-57% by the 0.03 μm (colloid fraction)-filter. Plutonium behavior during microfiltration of the 1:10 and 1:1000 treatments, where citrate metabolism was moderate, suggests that biosorption of the Pu-citrate complex alone is not responsible for removing Pu from solution since it remained in soluble form (>93%).

III. BACTERIAL DISSOLUTION OF ACTINIDES IN NTS SOIL

Characterization of Pu-contaminated Nevada Test Site (NTS) soil. Plutonium

Table 2. Isotopic activity in NTS soil.

Isotope	NTS Soil	
	(nCi/g)	($\mu\text{g/g}$)
U 233/234	< 0.18	< 6.1×10^{-2}
U 235/236	< 0.15	< 3.6×10^1
U 238	< 0.14	< 4.2×10^2
Am 241	3.8 ± 0.7	1.1×10^{-3}
Pu 238	0.44 ± 0.02	2.6×10^{-5}
Pu 239/240	69.2 ± 6.5	1.1×10^{-1}

contaminated soil (HP-11) was obtained from Area 11 soil of the Double Track test shot area at the NTS and had a gross activity of 50 nCi/g. The individual alpha components of the total activity were determined and are given in Table 2. The beta-emitter Pu-241 has also been detected but was not quantified.

Mineral content and association of Pu in NTS soil.

Minerals present in the soil were determined using μ -X-ray diffraction measurement on beamline X7A at the National Synchrotron Light Source (NSLS). The predominant minerals consisted of various forms of iron oxides and aluminosilicates. The uranium was present in the soil as hexavalent form associated as the minerals schoepite ($\text{UO}_3 \cdot 2\text{H}_2\text{O}$) and liebigite ($\text{Ca}_2\text{UO}_2(\text{CO}_3)_3 \cdot 11\text{H}_2\text{O}$). Association of Pu in the soils was attempted on beamline X27A. However, the Pu signal was below the detection limit of the methods (<1 μg).

Synchrotron scanning transmission X-ray microscopy (STXM) analysis was performed by Drs. D. Shuh, P. Nico and T. Tyliszczak at the Advanced Light Source (ALS), Berkeley, CA. The “as received” soil sample was analyzed on the Molecular Environmental Sciences beamline

ALS-MES 11.02. The image of the soil presented in Figure 9A is a 4 μm x 4 μm spot size obtained at an energy of 800 eV. Figure 9B shows the image of Pu obtained using a resolution of <100 nm). The Pu distribution is localized to a small area of the sample (approx. 500 nm) and suggests it is present as polymeric form.

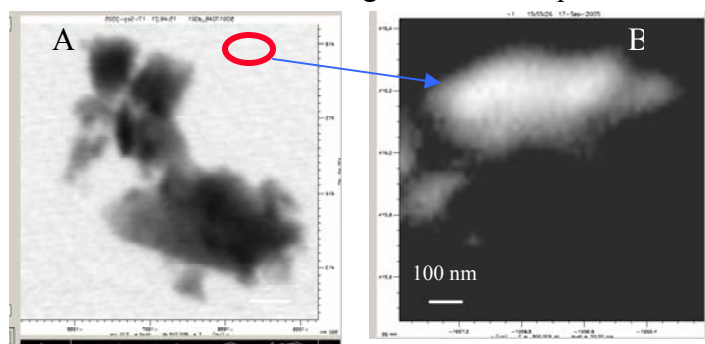


Figure 9. Synchrotron scanning transmission X-ray microscopy for NTS soil showing particles (A) and Pu-containing region (B).

Effect of bacterial activity on mobilization of Pu, Am, and U in NTS soil.

The effect of bacterial activity on the mobilization of radionuclides in NTS soil is presented in Figure 10. Under both aerobic and anaerobic conditions the degradation of glucose occurred with decrease in pH to 5.2

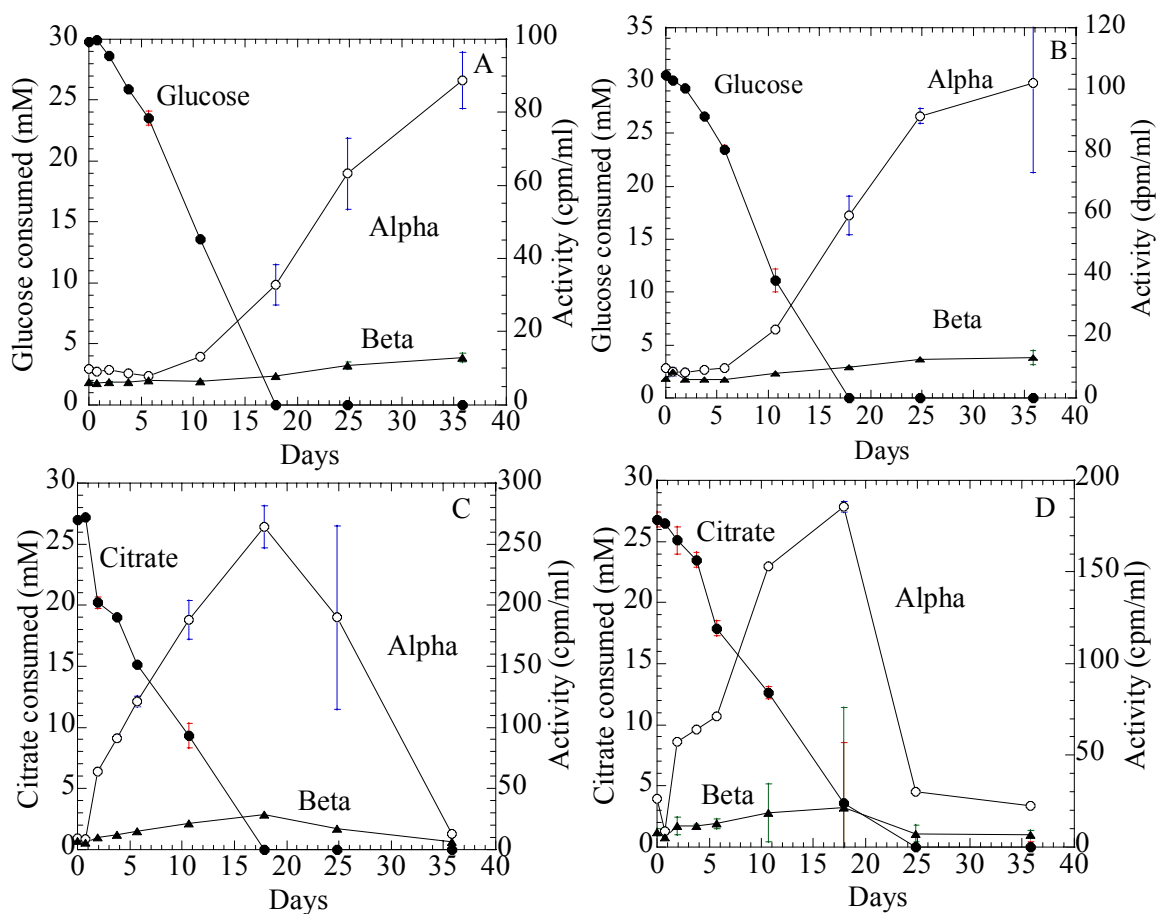


Figure 10. Effect of carbon utilization by indigenous bacteria on the mobilization of actinides.

for the aerobic sample and 4.83 for the anaerobic sample and an increase in actinide activity in solution. However, the appearance of the actinide in solution was concomitant with decrease in pH and not related to consumption of glucose (Fig. 10A and 10B). This indicates that the

predominant mechanism for alpha and beta release into solution is due to the slightly acidic pH of the spent medium. In addition, the alkaline nature of the soil suggests that mobilization may be due to dissolution of carbonate species present in the soil.

During the aerobic and anaerobic metabolism of citric acid both alpha and beta activity increase in solution up to 17-d. However with increase in time the activity begins to decrease (Figure 10C and 10D). This loss of activity in solution coincides with the complete utilization of citric acid by the bacteria. There is only slight decrease in pH during the experiment to 7.7 in the presence of aerobic bacteria and 7.4 in the presence of anaerobic bacteria. This observation suggests that citric acid forms a soluble complex with the actinide and that its removal due to bacterial metabolism releases the actinide, resulting in its precipitation. These results also suggest that the type of carbon source will have different effect on microbial mobilization of actinides.

INFORMATION ACCESS

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10. Francis, A.J. Dodge, C.J., and J.B. Gillow. Biotransformation of plutonium complexed with citric acid. *Radiochim. Acta*, 94: 731-737.

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17. Francis, A.J. 2006. Microbial Transformations of Radio nuclides and Environmental Restoration Through bioremediation. In Proceedings of the DAE_BRNS Symposium on Emerging Trends in Separation Science and Technology SESTEC2006. Eds. P.K. Mohapatra, R.M. Sawant, B. Venkataramani, and V.K. Manchanda. Sept 29- Oct 1, 2006. pp.42-51. Organized by Board of Research in Nuclear Sciences, Department of Atomic Energy

INVITED TALKS

1. Francis, A.J. "Microbial Transformations of Actinides and Environmental Restoration through bioremediation", Harry Reid Center, University of Las Vegas Nevada, February 18, 2005.
2. Francis, A.J. "Microbial Transformation of Radioactive Waste" NYU Graduate School of Journalism Science and Environmental Reporting Program, April 22, 2005.
3. Francis, A.J. "Microbial Transformations of Actinides and Environmental Restoration Through Bioremediation." Special Topic Lecture - ACS sponsored Summer School in Nuclear Chemistry at BNL June 27, 2005.
4. Francis, A.J. Microbial Transformations of Actinides in Transuranic and Mixed Wastes and its Implications on Radioactive Waste Disposal. Paper presented at the Actinides 2005 International conference, July 4- 8, 2005, Manchester, UK.
5. Francis, A.J. Biotransformation of Uranium Associated with Organic Ligands. Paper presented at the Uranium Mining and Hydrogeology VI (UMH IV) International Conference, September 11-14, 2005, Freiberg, Germany.

6. Francis A.J., C.J. Dodge, and J.B. Gillow. Biotransformation of plutonium complexed with citric acid. Paper presented at the 10th International Conference on Chemistry and Migration Behavior of Actinides and Fission Products in the Geosphere "Migration 2005" September 18-23, 2005, Avignon, France.
7. Francis, A.J. "Microbial Transformations of Radionuclides and Environmental Restoration Through Bioremediation" at the IUPAC sponsored 2nd International Symposium on Green/Sustainable Chemistry, held at the University of Delhi, India. January 10-13, 2006,
8. Francis, A.J. " Microbial Transformations of Radionuclides in Transuranic and Mixed Wastes" at the Indira Gandhi Center for Atomic Research (IGCAR), Kalpakkam, Chennai, Tamil Nadu. January 23-24, 2006.
9. Francis, A.J.; C.J. Dodge; J.B. Gillow. Pu-Futures – The Science 2006 Pacific Grove, California, July 9-13, 2006. Invited talk Microbial Transformations of Plutonium.
10. Francis, A.J. "Microbial Transformations of Actinides and Environmental Restoration Through Bioremediation." Special Topic Lecture - ACS sponsored Summer School in Nuclear Chemistry at BNL July 17, 2006.
11. Francis, A.J. SESTEC2006. Invited talk entitled "Microbial Transformations of Radionuclides and Environmental Restoration Through Bioremediation" at the SESTEC-2006 held at BARC, Mumbai, September 29 – October 1, 2006, organized by the Department of Atomic Energy (DAE) – Board of Research in Nuclear Sciences (BRNS) Biennial Symposium on "Emerging Trends in Separation Science and Technology" SESTEC-2006.
12. Francis, A.J. "'Emerging Trends in Separation Science and Technology'" September 13 – October 14 , 2006 at the Indira Gandhi Center for Atomic Research (IGCAR), Kalpakkam, Chennai, Tamil Nadu.
13. Francis, A.J. Microbial Transformations of Radionuclides Released from Nuclear Reprocessing Plants" at the International Symposium on Environmental Modeling and Radioecology" organized by the IES, Rokkasho, Aomori October 18-20, 2006.
14. Francis, A.J. International symposium on Advanced Science Research "Frontiers of Nuclear and Radiochemistry" ASR2006, organized by Advanced Science Research Center (ASRC), Japan Atomic energy Agency (JAEA), Tokai, Ibaraki, Japan. October 26-27, 2006. Invited talk "Microbial Transformations of Plutonium"
15. Dodge, C.J.; Francis, A.J.; Gillow, J.B. Uranium reduction by *Clostridia*. Presented at the Annual ERSD PI Meeting, April 5, 2006, DOE, Warrenton, VA.
16. Dodge, C.J. Center for Environmental Molecular Science. Remediation of wastes containing radionuclides and toxic metals using citric acid. Seminar, SUNY-SB, July 15, 2003.
17. Dodge, C.J. Remediation of wastes containing toxic metals and radionuclides using citric acid. CEMS Summer Undergraduate Research Program. June 24, 2005.
18. Nico, P.; Anastasio, C.; Dodge, C.; Fendorf, S.; Francis, A.J.; Hubbard, S.; Shuh, D.; Tomutsa, L.; Tufano, K.; Tyliszczak, T.; Werner, M.; Williams, K. Environmental Science Program at the Advanced Light Source. Presented at the Annual ERSD PI Meeting, April 5, 2006, DOE, Warrenton, VA.